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Facial Motor Neuron Migration Advances

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Abstract

During development, the migration of specific neuronal subtypes is required for the correct establishment of neural circuits. In mice and zebrafish, facial branchiomotor (FBM) neurons undergo a tangential migration from rhombomere 4 caudally through the hindbrain. Recent advances in the field have capitalized on genetic studies in zebrafish and mouse, and high-resolution time-lapse imaging in zebrafish. Planar cell polarity signaling has emerged as a critical conserved factor in FBM neuron migration, functioning both within the neurons and their environment. In zebrafish, migration depends on specialized 'pioneer' neurons to lead follower FBM neurons through the hindbrain, and on interactions with structural components including pre-laid axon tracts and the basement membrane. Despite fundamental conservation, species-specific differences in migration mechanisms are being uncovered.

Introduction

The development of the vertebrate brain is a complex process, in which the construction of brain architecture and intricate neural circuitry are effectively coordinated. One aspect of this process is the migration of neurons from their birth-places in the ventricular zone, to establish laminar or nuclear structures. Defective neuronal migration underlies several devastating neurological syndromes including lissencephalies (see article in this issue), and intractable forms of epilepsy [1]. Local radial migration is common, but in addition, specific neuronal types undergo longer distance tangential migrations, orthogonal to the plane of the neuroepithelium [2-4]. Here we focus on the tangential migration of FBM neurons, where extensive characterization is providing insights that may be more broadly applicable.

The FBM neurons form the motor component of the facial (VIIth cranial) nerve, which innervates second pharyngeal arch derivatives including facial muscles. Damage or deficits to the facial nerve are associated with syndromes and neuropathies such as Bell's Palsy [5] and Möbius syndrome [6]. Cranial motor neurons originate in specific rhombomeres of the segmented vertebrate hindbrain ([7]; Fig. 1), and follow stereotyped migration pathways to coalesce into motor nuclei. Caudally-directed migration of FBM neurons occurs in humans, mice and fish, but is minimal in chickens [7,8]. FBM neurons are born at the ventricular surface of ventral rhombomere 4 (r4) and extend axons via an r4 exit point into the facial

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nerve (Fig. 1). The FBM neuronal cell bodies migrate caudally adjacent to the medial floor plate into r6 (mouse) and r6/7 (zebrafish). In all species, the caudal migration appears to be a somatal translocation, independent of guidance by radial glia. The position that the neurons occupy during their caudal migration differs between zebrafish and mouse: in zebrafish, caudal migration follows a brief ventral migration to the pial surface of the neural tube (Fig. 1D, E), whereas in mice the neurons migrate closer to the ventricular surface [11,12] (Fig. 1F). Upon reaching their destination, FBM neurons undergo a lateral migration, away from the floor plate, to form nuclei (reviewed by [9,10]). In mice a final phase of radial migration is also required, from the ventricular to the pial surface, which is disrupted by mutations in *reelin* and *Dab1* [13] and likely occurs along radial glia. During migration, the facial motor axons are laid down behind the migrating neurons, forming a loop around the abducens nucleus (VIth nerve) to produce the internal genu (bend) of the facial nerve.

The role of intrinsic and extrinsic factors in FBM neuron migration

The migratory capacity of FBM neurons is an aspect of their regional identity, governed by an interplay between intrinsic and extrinsic factors (summarized in Table 1). Transcriptional regulatory networks specify FBM neurons, with anteroposterior and dorsoventral patterning information converging via *Hox* and *Nkx2.2* genes to regulate in particular the expression of *Phox2b* [14]. *Hoxb1*, which is highly expressed in r4, is an important player, conferring migratory capacity on FBM neurons via downstream targets [12,15-19]. Mice mutant for the *Hoxb1* targets *Gata3* and *Tbx20* lack FBM migration, and knock-in approaches have revealed a specific role for *Phox2b* [19]. Rohrschneider and colleagues [20] identified zebrafish *prickle1b* (*pk1b*) as a *Hoxb1a*-regulated gene with elevated expression in FBM neurons. Ectopic expression of *pk1b* in r2-derived trigeminal BM neurons (which normally migrate only laterally; Fig. 1) is sufficient to cause these cells to migrate caudally [21]*, implying that neuron-intrinsic changes in gene expression can confer tangential migratory properties. Mapp et al. [21]* also demonstrated that *Pk1b* functions in the nuclei of FBM neurons to localize the transcriptional repressor *Rest*, suggesting that *Rest* represses terminal maturation genes to maintain the competence of FBM neurons to respond to migration cues [21]*[22].

Intrinsic factors render FBM neurons competent to respond to external cues via regulation of a repertoire of receptors. While understanding of this regulation is limited, the neuronal membrane proteins *Tag1*, *Ret* and *Cadherin8* are expressed during specific phases of mouse FBM migration, and deletion of transcription factor *Ebf1* leads to incorrect spatial regulation of these molecules [23]. Deletion of mouse *Tbx20* also alters expression of molecules in the planar cell polarity (PCP) pathway [9]. In addition, several receptors play important roles in FBM neuron migration, via interactions with their environmental ligands [24-26] (Table 1). For example, *Vegf164* expressed in the caudal hindbrain exerts a chemoattractive effect on FBM neurons via *Nrp1* [25], and *Wnt5a*-mediated chemoattraction may also be instructive in the caudal movement [27]. Whereas these receptors and ligands provide directional information, a variety of molecules and structural features also provide permissive cues; as yet the interactions between these factors remain elusive.

PCP signaling plays a key role in FBM migration

Recent studies have revealed the critical importance of PCP signaling in orchestrating FBM neuron migration. PCP signaling has been primarily studied in the context of the polarization of epithelial tissues (Box 1). Genetic screens in zebrafish using the *isl1:GFP* transgenic line [28] (Fig. 1) and knockout analyses in mice, have revealed that FBM neuron migration requires multiple PCP molecules including *Frizzled3a*, *Vangl2*, *Celsr2*, and the polarity molecule *Scrib* [27,29-33]. Within non-neural tissues, vertebrate PCP signaling

typically involves an environmental influence of non-canonical Wnts, but within the nervous system the role of extrinsic Wnt ligands remains controversial. For example, FBM neuron migration is not disrupted in zebrafish mutant for non-canonical *wnt5b* and *wnt11*, or the *glypican4/6* positive modulator of PCP [10]. By contrast, in the mouse Vivancos and colleagues [27] found that Wnt5a shows an intriguing rostral^{low} to caudal^{high} step gradient of expression across r4 to r6 consistent with a chemoattractive effect on FBM neurons. Using hindbrain explant assays they showed that beads carrying Wnt5a or Wnt7a are chemoattractant for mouse FBM neurons. Conversely, Sfrps (Secreted frizzled related proteins), which attenuate Wnt/Fz signaling, block FBM neuron migration. Consistent with an in vivo role for Wnt signaling, Wnt5a mutants show partial defects in the FBM migration, although Wnt7a mutants do not.

Although the role of Wnts requires clarification, extrinsic functions of PCP molecules have been confirmed by zebrafish cell transplantation experiments (Fig. 2A-C). Non-cell autonomous function of PCP molecules such as Frizzled3a, Celsr2, Vangl2 and Scrib [30,31,34] (as well as the adhesion molecule N-Cadherin/Cdh2 [35]*) has been shown to maintain structural integrity of the neuroepithelium. Neuroepithelial cohesion apparently ensures that zebrafish FBM neurons are ‘excluded’ and move apicobasally close to the basement membrane during migration, where they are juxtaposed with other guidance cues. Walsh and colleagues [36]** realized that demonstrations of non-cell autonomous PCP function can be confounded by collective migration or ‘community effects’ in which wild-type FBM neurons influence behavior of adjacent mutant neurons [24]. Using an elegant approach to avoid collective migration ([36]**; Fig. 2D-F), these authors demonstrated that PCP molecules act both in the FBM neurons and in their environment. This study also demonstrated a role for Vangl2 and Scrib in conferring rostrocaudal polarity on the specialized midline floor plate cells ([36]**; Fig. 1).

We propose key differences in PCP action between zebrafish and mouse. Importantly, murine FBM neurons are not excluded from the neuroepithelium to migrate adjacent to the pial surface [12]. Further, there are more published examples of murine PCP components expressed in FBM neurons than in their environment [12,37], implying cell-autonomous functions. Consistent with this, conditional knockout analysis of *Celsr3* has confirmed function within the FBM neurons, although similar approaches indicate *Celsr1* functions non-cell autonomously [33]. Deeper understanding of PCP in mouse FBM neuron migration will require additional conditional knockout studies.

Downstream signaling and the cytoskeleton

Ultimately, polarized environmental guidance cues acting via asymmetric receptor assemblies in FBM neurons must influence the cytoskeleton. One attractive model suggests the centrosome functions at the leading neuronal edge to transmit signals, leading to advancement of the nucleus via a peri-nuclear cage of microtubules [38]. Actomyosin contractility assists the movement that ‘drags’ the nucleus forward; mice lacking MyosinIIB have disrupted FBM migration [39]. Yet currently there is little information as to how PCP or other molecular mechanisms regulate cytoskeletal components. In other contexts, PCP signaling operates via intracellular mediator Dishevelled (Dsh/Dvl; see Box 1), but it is unclear whether this applies in FBM neuron migration. Glasco and colleagues [32]* explored the role of Dsh/Dvl in both zebrafish and mice, and found that loss of function failed to disrupt FBM migration [34], although a role for mouse Dvl3 remains untested. Despite the equivocal role of Dsh signaling, it appears that typical Wnt/PCP downstream effectors such as Rac and Rho still act via JNK and RhoKinase (ROCK) respectively. Vivancos and colleagues [27] demonstrated that inhibition of JNK, ROCK or MyosinII disrupts mouse FBM neuron migration in explants. These pathways are likely regulated at

least in part by Wnt/PCP signaling; a possible link is provided by MAGI-3, a scaffold protein that can bind Frz receptors and Vangl2, and which can signal via Rac and JNK [40]. Recent insight was provided by Walsh and colleagues [36]** who identified the zebrafish PCP effector Nance-Horan syndrome-like1b (Nhs11b). Nhs11b is necessary for FBM neuron migration, and is expressed at elevated levels in the migrating neurons, where it localizes to the leading edge of membrane protrusions. Given that Scribble plays a role in Rac activation at the leading edge and binds directly to Nhs11b, this would provide a direct link between PCP signaling and the actin cytoskeleton to regulate migration [36]**. These mechanisms are likely a very partial view of cytoskeletal regulation in FBM neurons; MyosinII and microtubules also participate, with the latter likely to be regulated by Cdk5, as in Cdk5-deficient mice FBM neurons fail to migrate out of r4 [41]. It has also been proposed that Vegf signaling might link directly to Cdk5 via Nrpn1 [25], perhaps suggesting Cdk5 as an integrator of signaling.

FBM neuron migration in zebrafish depends on neuron-neuron interactions and the basal lamina

Neuron-to-neuron interactions clearly play an important role during FBM migration, although the nature of such contact-mediated signaling is poorly defined. Wanner and Prince [42]** recently reported that in zebrafish the first ‘pioneer’ FBM neuron to migrate leads other neurons in their earliest phase of migration from r4 into r5. Ablation of the pioneer or its trailing axon blocks follower FBM neuron migration [42]**, suggesting physical interactions between pioneer and followers are critical. These interactions depend in part on adhesion molecule N-cadherin/Cdh2, with Cdh2 depletion resulting in stalled follower neuron migration [42]**. Pioneer neuron migration is independent of Cdh2 function or obvious local physical cues [35] [42]**, and time-lapse imaging revealed pioneer-specific behaviors, suggestive of active path-finding [42]**.

A subsequent phase of zebrafish FBM neuron migration, from r5 to r6, relies on the interaction between FBM neurons and the medial longitudinal fasciculus (MLF; Fig. 1). Migrating FBM neurons stall in response to mechanical blockage of MLF extension, and depletion of Cdh2 reduces interactions between the MLF and the neurons [42]**. Interestingly, disruption of adhesion molecule Cntn1/Tag1 also causes a partial block of FBM neuron migration [43]; Tag1 is expressed in the FBM neurons but also in the MLF, and is required for its normal fasciculation [44]. In summary, zebrafish FBM neurons utilize adhesion-dependent interactions with local axon tracts to migrate through the hindbrain. Whereas these mechanisms have not been extensively investigated in mouse, it is noteworthy that both Cadherin8 and Tag1 can mediate homophilic interactions between neurons and/or axon tracts and are expressed during distinct phases of FBM neuron migration [23]. However, the more dorsal position of migrating FBMs in mouse (Fig. 1) physically separates the neurons from the MLF, suggesting an MLF-dependent mechanism does not operate [45].

As a result of the pial location of FBM neurons in the zebrafish, the basement membrane plays an important role in facilitating their migration. Apicobasal polarity molecules of the PAR-aPKC complex establish basal epithelial structures, and live imaging has confirmed that zebrafish FBM neurons migrate very close to the basal (pial) surface of the hindbrain [11]*. Disruption of PAR-aPKC molecules in zebrafish neuroepithelium not only disrupts FBM neuron migration, but remarkably allows the neurons to migrate through the basement membrane to exit the hindbrain [11]*. The Laminin-rich basement membrane provides a substrate for neuronal migration, as ectopic localization of FBM neurons can be phenocopied by disrupting *lamininA1* function [11]*[46]. The conserved molecule Par6 may interact with Scribble in specifying apicobasal polarity, and provide a common mechanism

in both zebrafish and mouse [47]. Par6 also interacts with ROCK to regulate MyosinIIB, thus converging with PCP-dependent signaling via ROCK and JNK [48].

Conclusions

Recent studies have revealed that tangential FBM neuron migration is highly dependent on PCP-signaling acting both intrinsically and extrinsically. In addition, it depends on a variety of interactions between FBM ‘pioneer’ and follower neurons, axon tracts and the basement membrane, with important roles played by Cdh2 and Laminin α 1. Although rostrocaudal and apicobasal polarity of the neuroepithelium and floor plate are emerging as important factors, it is as yet unclear how these two phenomena are integrated to generate caudal migration. A more complete understanding of the subcellular distribution of PCP proteins in both neurons and neuroepithelial cells will help us to understand how PCP provides migratory cues. However, it will also be important in the future to combine these PCP-focused studies with those on other guidance systems such as Vegf and Cxcl12, and in turn to relate these mechanisms to neuronal adhesion systems. Understanding how asymmetric cues are transduced to influence the centrosome and machinery at the leading edge remains a challenge, but Nhs11b now provides a promising link with the PCP pathway. The prospect of live imaging of labeled proteins and structures in transgenic zebrafish will provide an unparalleled opportunity to unravel this complexity in the future.

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which point they accelerate and reorient their centrosomes to the posterior of the neurons. Disruption of PAR-aPKC complex molecules disrupts neuron migration and allows the neurons to escape the hindbrain by migrating ventrally through holes in the Laminin-rich basement membrane; mutation of *laminin1* phenocopies this result. The authors conclude that the Laminin-rich basement membrane functions both as a substrate for migration and as a boundary to ensure FBM neurons remain on their proper migratory route.]

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collective mode of migration (dependent on interactions between neurons) and a PCP-dependent mode of migration that relies on interactions between neurons and their polarized environment (Fig. 2). Importantly, these transplantation experiments confirm the requirement for PCP signaling both in the environment and in the neurons themselves. Nhs11b encodes a WAVE-homology domain that in other systems interacts with the WAVE regulatory complex to control cytoskeletal dynamics; this, together with the demonstration that Nhs11b protein localizes to FBM neuron membrane protrusions, strongly suggests it functions downstream of PCP signaling to mediate mechanical aspects of FBM neuron migration.]

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Highlights

- Facial branchiomotor neurons migrate tangentially through the vertebrate hindbrain
- Genetics and imaging are providing new insights into FBM neuron migration processes
- Planar cell polarity plays both intrinsic and extrinsic roles in neuron migration
- Pre-laid axon tracts provide structural cues for FBM neurons to migrate along
- Migration occurs in both mouse and zebrafish, but mechanistic details differ

Box 1: Planar cell polarity

PCP molecules function in epithelia to polarize cells via establishment of polarized sub-cellular differences that are communicated from cell to adjacent cell to influence an entire field. This molecular polarity can then be translated into morphological asymmetries via downstream effectors that interact with the cytoskeleton. Note that the roles of Wnt ligands and the intracellular mediator Dsh in FBM neuron migration remain unclear. Nhs11b has recently been identified as a zebrafish PCP effector that interacts genetically and physically with Scribble (Scrb) in the FBM neurons.

Figure 1. Zebrafish and mouse facial branchiomotor neuron migration

A. Dorsal view of zebrafish branchiomotor neurons (BMNs) in an *Tg[isll:GFP]* (red) transgenic embryo, 48hpf. Rhombomere (r) number is indicated. B. Schematic rendering of zebrafish (48hpf) and mouse (12.5 dpc) BMN organization in dorsal view, anterior to the top. FBM neurons in red, other BM neurons in blue, abducens motor neurons in black; ov, otic vesicle; fp, floor plate (yellow). C. Dorsal view of a mouse embryo labeled by in situ hybridization with *Islet-1*, highlighting the trigeminal (in r2) and FBM neurons at 12.5 dpc. D. Transverse section of a zebrafish embryo at the level of r5 at 24hpf, dorsal up. FBM neurons (red) are in close contact with the MLF (green, arrow). The pial or basal surface (p/b, dashed white line), ventricle or apical surface (v/a, dashed blue line), and floor plate (fp, yellow) are outlined. E. Schematic of the ventral region of the zebrafish neural tube highlighted in D (gray box). FBM neurons (red) migrate caudally along the MLF (green) at the pial surface of the neural tube (arrow), adjacent to the floor plate (fp, yellow) and between endfeet of the neuroepithelial cells (orange) of the neural tube. The ventricle/apical surface is highlighted in blue (arrowhead) and the pial/basal surface is in black (arrow). F. Schematic of FBM neuron (red) migration through the mouse hindbrain. Neurons migrate caudally (1) close to the ventricular surface; this is followed by lateral (2) and radial (3) migration in r6. The ventricle/apical surface is highlighted in blue (arrowhead) and the pial/basal surface is in black (arrow), floor plate in yellow.

Fig. 2. Zebrafish cell transplantation experiments reveal intrinsic and extrinsic roles for PCP signaling in FBM neuron migration

Cell transplantation experiments in the zebrafish are used to generate genetically chimeric specimens that combine mutant and wild-type cells, providing a powerful approach to address cell autonomy of gene function. Schematics compare localization of donor-derived (red) and host-derived (green; *Tg[Islet-1:GFP]*) FBM neurons in a variety of such experiments. A. When donor and host neurons are both wild-type (WT) migration is normal. B. WT donor-derived neurons do not migrate through a PCP-deficient environment [30,31,34]. C. PCP-deficient donor-derived neurons can migrate through a WT environment [30,31,34]. D. Pk1b is required cell autonomously for FBMN migration [21]. E. WT donor-derived neurons can migrate, albeit with reduced efficiency, through a Pk1b-deficient environment (consistent with the neuron- intrinsic role of Pk1b) [20]. F. PCP-deficient neurons do not migrate through a Pk1b-deficient environment, due to lack of collective migration, revealing a cell-autonomous role for PCP signaling intrinsic to FBM neurons and confirming the existence of both a ‘collective’ migration mode and a PCP-dependent migration mode [36].

Table1
Intrinsic and extrinsic factors that function in FBM neuron migration

A non-comprehensive summary focused on molecules discussed in the text, note that multiple PCP pathway molecules also play roles in FBM neuron migration, see Box 1.

	Molecule	Animal; Experiment; Imaging; Reference	Expression	Phenotype
Intrinsic	• Met	• Zebrafish; MO knockdown; <i>Tg[isl1GFP]</i> ; [24]	• Expressed in FBM neurons during their caudal migration	• A defect in caudal migration; 60% of FBMNs fail to reach r6 (see Hgf1/2 phenotype)
	• Nrp1	• Mouse; gene knockout; ISH; [25]	• Expressed in FBM neurons as during their caudal and lateral migration	• Caudally migrating FBM neurons form multiple streams and undertake a premature radial migration forming a distorted, 'dumbbell' nucleus (see Vegf164 phenotype)
	• Cxcr4/ Cxcr7	• Zebrafish; MO knockdown; <i>Tg[isl1GFP]</i> ; [26, 49]	• Cxcr4 expression in FBM neurons commences as they begin to migrate caudally and stops as they start to migrate laterally • Cxcr7 is initially expressed in r3 and r5 and as the FBM neurons begin to migrate its expression decreases in r3 and increases in r6	• Knockdown of Cxcr4 causes an equal distribution of FBMNs in r4, r5 and r6 • Knockdown of Cxcr7 leads to an accumulation of FBMNs in r5 • A combined knockdown of Cxcr4 and Cxcr7 produces a third phenotype, in only 50% of cases, where the FBMNs accumulate within r4 and r5
	• Dab1	• Mouse; gene knockout; ISH/ IHC; [13]	• Expressed by the majority of migrating FBM neurons	• Disorganisation and dispersion of the facial nucleus (see Reelin phenotype)
Extrinsic	• Hgf1/2	• Zebrafish; MO knockdown; <i>Tg[isl1GFP]</i> ; [24]	• Expressed at low levels throughout the hindbrain and in the ventral tissues underlying the migrating FBM neurons	• Double Hgf1/2 knockdown produces a defect in caudal migration, with 60% of neurons failing to reach r6 (see Met phenotype) • Single Hgf1 or Hgf2 knockdown produces a milder phenotype
	• Vegf164	• Mouse; downregulation of Vegf164; ISH; [25]	• Expressed in the midline, in paired dorsomedial stripes running the length of the hindbrain and in the final location of the facial nucleus	• Caudally migrating FBMNs form multiple streams and undertake a premature radial migration forming a distorted, 'dumbbell' nucleus (see Nrp1 phenotype) • There is also some 'scattering' of neurons on the pial side
	• Sdf1a	• Zebrafish; MO knockdown; <i>Tg[isl1GFP]</i> ; [26]	• Initially expressed broadly in the hindbrain; as the FBMNs begin to migrate its expression is decreased first in r5 followed by r4 and r6	• Failure of caudal migration with the neurons projecting axons laterally out of r4 and r5
	• Reelin	• Mouse; gene knockout; ISH/ IHC; [13]	• Expressed in the final location of the facial nucleus	• Disorganisation and dispersion of the facial nucleus (see Dab1 phenotype)
	• Wnt5a/Wnt7a	• Mouse; migration assay; ISH/ IHC; [27]	• Wnt5a is expressed in the hindbrain in a rostral ^{low} to caudal ^{high} , rhombomere dependent, stepwise gradient • Wnt7a is expressed throughout the hindbrain and particularly concentrated in the ventricular zones and neuroepithelium	• Beads soaked in Wnt5a/ Wnt7a and placed in rostral r4 acted as chemoattractants for FBMNs
	• Laminino1	• Zebrafish; mutation; <i>Tg[isl1GFP]</i> ; [46]	• -Expressed in the basal lamina	• Loss of laminino1 causes disruption of FBMN migration, with some neurons passing through the basement membrane to exit the neuroepithelium
	• Cdh2	• Zebrafish; MO knockdown/ gene mutation; IHC; [35]	• Cdh2 is expressed ubiquitously throughout the hindbrain	• Both MO knockdowns and mutant embryo's show a failure of FBMNs to leave r4 and in the more severe phenotypes there is a fusion of the FBMN clusters over the midline

FBMN: facial branchiomotor neuron, MO: morpholino oligonucleotide, ISH: in situ hybridisation, IHC: immunohistochemistry